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Didymodon manhanensis (Pottiaceae, Bryophyta), a new species from Inner Mongolia steppe, China and its phylogenetic position, based on molecular data

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Abstract

Inner Mongolia steppe is one of the suitable habitats for *Didymodon* species and a new species, *Didymodon manhanensis* C. Feng & J. Kou from Manhan Mountain in semi-arid region in Inner Mongolia, China is described and illustrated. It is characterised by leaves incurved and slightly twisted when dry, spreading when moist, narrowly lanceolate from an ovate base; subulate and fragile leaf apices; distally bistratose leaf margins that are recurved in proximal 2/3–3/4; excurrent costa with guide cells in 2–3 layers and without ventral stereids; smooth laminal cells and red KOH laminal colour reaction. Our morphological analyses and molecular results, based on DNA sequences of ITS, *rps*4 and *trn*M-*trn*V, confirm that *D. manhanensis* belongs to a group that includes *D. obtusus* J. Kou, X.-M. Shao & C. Feng and *D. daqingii* J. Kou, R.H. Zander & C. Feng. This new species is compared with similar species and its phylogenetic position and ecology are discussed.

Keywords

Asia, Manhan Mountain, phylogenetic analysis, taxonomy

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Introduction

Inner Mongolia, situated in Inner Eurasia, is located in the northern part of China and presents a strip distribution from the northeast to the west. The district habitats are temperate continental monsoon climate. The annual mean temperature is 8 °C, which increases from east to west and the annual precipitation is 35–530 mm, which decreases from southeast to northwest (Miao 2017). The area of grassland accounts for 60% of the whole Inner Mongolia and more than one-quarter of the total area of grassland in China. The grassland in Inner Mongolia is divided into three types: meadow steppe formed by, for example, *Stipa baicalensis* Roshev, *Leymus chinensis* (Trin.) Tzvelev; typical steppe formed by, for example, *Stipa grandis* P. Smirn., *Stipa krylovii* Roshev, *Leymus chinensis* (Trin.) Tzvelev and desert steppe formed by, for example, *Stipa klemenzii* Roshev, *Stipa glareosa* P.A. Smirn., *Stipa breviflora* Griseb (Hua et al. 2021). The main vegetation types of the steppes present distinct zonal features (Wu et al. 2005). The Inner Mongolia steppe is a suitable habitat for the *Didymoodn* Hedw. species and several new species were recently discovered (e.g. Kou et al. 2016a; Kou et al. 2019; Feng et al. 2022).

The taxonomy of genus *Didymodon* is complicated, involving the differentiation from related genera, such as *Barbula* Hedw. and the circumscriptions of its infrageneric sections (Zander 1993; Zander 2007; Zhang et al. in press). A recent important event associated with *Didymodon s. lat.* was the split of the genus into seven smaller genera: *Aithobryum* R.H. Zander, *Didymodon s. str.*, *Exobryum* R.H. Zander, *Fuscobryum* R.H. Zander, *Geheebia* Schimp., *Trichostomopsis* Card. and *Vinealobryum* R.H. Zander, based on macro-evolutionary analysis and the dissilient genus concept applied (Zander 2013; Zander 2019). Although initially this revolutionary concept was considered unnecessary or unsupported (Blockeel and Kučera 2019), it has later been supported by molecular phylogenetic data, but with some alterations (Jiménez et al. 2022; Zhang et al. in press) and has gained acceptance by some other authors (e.g. Kou and Feng 2018; Osman et al. 2021; Feng et al. 2022). During our continuous investigations of xerophilic mosses, especially Pottiaceae Hampe, in China (e.g. Feng et al. 2016a, b; Kou et al. 2016b, 2018, 2019; Kou and Feng 2018), many specimens have been collected from different provinces. Amongst them, two samples collected from Manhan Mountain in Inner Mongolia of *Didymodon* s. lat. from stony habitats are different from species previously reported in the area (Li et al. 2001). They mostly resemble *Didymodon obtusus* J. Kou, X.-M. Shao & C. Feng. To clarify their taxonomic identity, we conducted phylogenetic analysis and confirm that these samples belong to the genus *Didymodon s. str.* (Zander 2013), but do not match with any species known in the genus. Here, we describe this unknown moss as a new species.

Materials and methods

Morphological observations

Over 3000 specimens of the genus *Didymodon s. lat.* were examined during our revision of Pottiaceae in China. More than 50 field investigations were conducted in past

years and the specimens included in this study were housed in the Herbaria at IFP, KUN and NMAC. Microscopic examinations and measurements were taken with a ZEISS Primo Star light microscope and photomicrographs were obtained with a Canon EOS 70D camera, mounted on this microscope. Specimens were examined in 2% potassium hydroxide (KOH). Three plants were dissected from each collection and, for each shoot, every possible structure from the gametophyte had to be examined and a record kept of what was found for each individual species. Specific morphological and anatomical features of taxonomic importance were assessed mainly following Zander (1993). Leaves were always taken from the upper and middle parts of the stem and cross-sections were made in the middle part of the stem. Measurements of leaf width were taken at the base, mid-leaf and upper part. Cross-sections were made at mid-leaf.

Phylogenetic analyses

To test the phylogenetic position of the new species, two specimens collected from Manhan Mountain were sampled. Due to its great similarity with *D. obtusus* and *Didymodon daqingii* J. Kou, R.H. Zander & C. Feng, the isotypes of the two species were added to the dataset. We employed one nuclear (ITS) and two chloroplast markers (*rps*4 and *trn*M-*trn*V), which had been used successfully in previous phylogenetic studies in *Didymodon* s. lat. and enabled the re-use of earlier results and easier interpretation of new data (Werner et al. 2004, 2005, 2009; Kučera and Ignatov 2015; Kučera et al. 2018; Ronikier et al. 2018; Jiménez et al. 2022; Zhang et al. in press). Phylogenetic trees are created and shown separately. The complete list with sample names and GenBank accession numbers is presented in Tables 1 and 2. DNA extraction, PCR amplification and sequencing procedure followed the protocols described by Wang et al. (2010).

The sequences were aligned by using MAFFT 7.222 (Kazutaka and Daron 2013) and then edited in BioEdit 7.0.1 (Hall 1999). The concatenation of individual *rps*4 and *trn*M-*trn*V fragments was performed by our custom Perl script. Phylogenetic analyses were performed by using the Bayesian Inference (BI) and Maximum Likelihood (ML) methods. MrBayes 3.2.6 (Ronquist et al. 2012) was used for BI analyses under the GTR substitute model. The following was used: two Markov Chain Monte Carlo (MCMC) searches were run for 10 million generations each, with a sampling frequency of 1000. The first 25% of the trees were discarded as burn-in. The convergence between runs in all cases dropped below

Table 1. New sequences used in this study, including taxa vouchers information and GenBank accession numbers.

Species	Voucher information	ITS	*rps*4	*trn*M-*trn*V
Didymodon manhanensis 4	China, Inner Mongolia, Chao Feng 2016060162	OL514237	OL450506	OL450515
Didymodon manhanensis a3	China, Inner Mongolia, Chao Feng 2016060176	OL514238	OL450507	OL450516
Didymodon obtusus	China, Tibet, Xiao-Ming Shao & Jin Kou 20140815037	OL514239	OL450508	OL450517
Didymodon daqingii	China, Inner Mongolia, Chao Feng 20170605032	OL514240	OL450509	OL450518

Table 2. Sequences from GenBank used in this study, including taxa and GenBank accession numbers.

Species	ITS	*rps4*	*trnM-trnV*
Acaulon triquetrum	MW398556		
Aloina rigida	MW398549		
Aloinella andina	MW398550		
Andinella churchilliana	MW398720		
Andinella coquimbensis	MW398711		
Andinella elata	MW398708		
Andinella granulosa	MW398714		
Andinella limensis	MW398710		
Andinella oedocostata	MW398733		
Andinella pruinosa	MW398726		
Barbula unguiculata	MW398553	HM147777	JQ890366
Bryoerythrophyllum recurvirostrum	MW398547	JQ890468	JQ890407
Bryoerythrophyllum rubrum	MW398548		
Chenia leptophylla	MW398561		
Cinclidotus riparius	MW398554		
Crossidium squamiferum	MW398558		
Didymodon acutus	AY437111	KP307551	KP307667
Didymodon alpinus	MW398606		
Didymodon andreaeoides	MW398768		
Didymodon anserinocapitatus	MW398649	KP307545	KP307640
Didymodon asperifolius	MW398594	JQ890472	KP307600
Didymodon australasiae (*Trichostomum australasiae*)	MW398737	KP307571	KP307651
Didymodon brachyphyllus (*Vinealobryum brachyphyllum*)	MW398817		
Didymodon buckii	MW398578		
Didymodon caboverdeanus	MW398607		
Didymodon californicus (*Vinealobryum californicum*)	MW398819		
Didymodon canoae	MW398584		
Didymodon cardotii	MW398729		
Didymodon challaensis (*Trichostomopsis challaensis*)	MW398748		
Didymodon constrictus	MW398613		
Didymodon cordatus	MW398664	KP307564	KP307668
Didymodon ditrichoides	MW398642		
Didymodon eckeliae (*Vinealobryum eckeliae*)	MW398826		
Didymodon edentulus	MW398685		
Didymodon epapillatus	MW398665		
Didymodon erosodenticulatus	MW398792	MF536597	MF536635
Didymodon erosus	EU835148	MF536609	MF536646
Didymodon fallax (*Geheebia fallax*)	MW398779	KP307552	KP307663
Didymodon ferrugineus (*Geheebia ferruginea*)	MW398796	MF536588	MF536625
Didymodon fragilicuspis	KP307482		
Didymodon fuscus	MW398689	KP307537	KP307601
Didymodon aff. *fuscus*		KP307546	KP307615
Didymodon gaochienii		KP307538	KP307658
Didymodon gelidus	MW398693		
Didymodon giganteus	MW398786	KP307548	KP307669
Didymodon glaucus	MW398612		
Didymodon guangdongensis (*Vinealobryum guangdongense*)	MW398657		
Didymodon hedysariformis	MW398582	KP307569	KP307629
Didymodon hengduanensis	MW398629		
Didymodon hegewaldiorum	MW398739		
Didymodon herzogii	MW398746		
Didymodon humboldtii	MW398667		
Didymodon icmadophilus	MW398632	KP307598	KP307604
Didymodon imbricatus	MW398646		

Species	ITS	*rps4*	*trnM-trnV*
Didymodon incrassatolimbatus	MW398572		
Didymodon incurvus	MW398680		
Didymodon insulanus (*Vinealobryum insulanum*)	MW398811		
Didymodon japonicus	MW398757		
Didymodon jimenezii	MW398622		
Didymodon johansenii	MW398589	KP307542	KP307662
Didymodon kunlunensis	MW398610		
Didymodon laevigatus	MW398618		
Didymodon lainzii	MW398575		
Didymodon leskeoides (*Geheebia leskeoides*)	MW398777	MF536604	MF536642
Didymodon luehmannii	MW398718		
Didymodon luridus	AY437098	MF536587	MF536624
Didymodon maschalogena	MW398615		
Didymodon maximus (*Geheebia maxima*)	MW398784	MF536591	MF536628
Didymodon mesopapillosus	MW398758		
Didymodon molendoides	MW398687		
Didymodon mongolicus	KU058175		
Didymodon murrayae	KP307513	KP307563	KP307650
Didymodon nevadensis	MW398730		
Didymodon nicholsonii (*Vinealobryum nicholsonii*)	MW398808		
Didymodon nigrescens	LC545516	KP307543	KP307611
Didymodon norrisii	MW398830	KP307585	KP307617
Didymodon novae-zelandiae	MW398769		
Didymodon obtusus	MW398666		
Didymodon occidentalis		KP307533	KP307599
Didymodon ochyrarum	MW398763		
Didymodon paramicola (*Trichostomopsis paramicola*)	MW398740		
Didymodon patagonicus	MW398675		
Didymodon perobtusus	KP307523	KP307539	KP307609
Didymodon revolutus (*Husnotiella revoluta*)	MW398569	JQ890471	KP307646
Didymodon revolutus var. *africanus*	MW398568		
Didymodon rigidulus	MW398602	KP307589	KP307647
Didymodon rigidulus var. *subulatus*	MW398672		
Didymodon rivicola	MW398599	KP30756	KP307607
Didymodon santessoni	MW398705		
Didymodon sicculus	MW398801	MF536606	MF536643
Didymodon sinuosus	MW398567	JQ890476	JQ890410
Didymodon spadiceus (*Geheebia spadicea*)	MW398795	MF536593	MF536631
Didymodon subandreaeoides	AY437108	KP307570	KP307630
Didymodon tectorum	MW398659		
Didymodon tibeticus	MW398638		
Didymodon tomaculosus	AY437114		
Didymodon tophaceus	MW398807	MF536607	MF536644
Didymodon tophaceus var. *anatinus*		MF536589	MF536626
Didymodon torquatus	MW398719		
Didymodon umbrosus (*Trichostomopsis umbrosa*)	MW398742		
Didymodon validus	MW398650		
Didymodon vinealis (*Vinealobryum vineale*)	MW398815	JQ890475	KP307606
Didymodon vinealis var. *rubiginosus*	MW398822		
Didymodon vulcanicus	MW398636		
Didymodon waymouthii	MW398770		
Didymodon wisselii	MW398655		
Didymodon xanthocarpus	MW398696	KP307534	KP307638
Didymodon zanderi	MW398585	KP307535	KP307621
Dolotortula mniifolia	MW398555		
Erythrophyllopsis andina	MW398546		

Species	ITS	*rps4*	*trnM-trnV*
Gertrudiella uncinicoma	MW398698		
Gertrudiella uncinicoma var. *serratopungens*	MW398701		
Guerramontesia microdonta	MW398543		
Hennediella heimii	GQ339750		
Hennediella polyseta	GQ339759		
Leptodontium excelsum	MW398545		
Microbryum curvicolle		JX679986	JX679936
Microbryum davallianum	MW398557		
Pseudocrossidium hornschuchianum	MW398551	JQ890481	JQ890420
Pseudocrossidium revolutum	MW398552		
Pterygoneurum ovatum	MW398560		
Sagenotortula quitoensis	GQ339761		
Stegonia latifolia	MW398559		
Syntrichia ruralis	MW398564	FJ546412	FJ546412
Tortula muralis	MW398562	JN581679	JQ890421
Tortula subulata	MW398563		
Triquetrella arapilensis	MW398544		
Tridontium tasmanicum	MW398750		

0.01. ML analyses were executed in IQ-TREE 1.6.3 (Nguyen et al. 2014) under the TPM3u+F+R3 (for cpDNA) and TIM3e+I+G4 (for ITS) substitute models, respectively, selected by the ModelFinder programme (Kalyaanamoorthy et al. 2017), based on the Bayesian Information Criterion (BIC) and 1000 fast bootstrapping replicates were used. The final obtained trees were visualised and edited in FigTree v.1.4.0 (Rambaut 2014).

Results

The chloroplast (cp) and ITS alignments comprised 1313 and 1364 nucleotide sites, respectively. The BI and ML phylogenetic trees have a consistent topology, although there are different levels of support depending on the method. Hence, only the topologies with branch lengths from the BI trees are presented, with added support from the ML method on the respective trees (Figs 3–4). Although the inference from analysed chloroplast regions (Fig. 3) and the ITS (Fig. 4) agrees in most aspects, the position of the new species is different between the two above phylogenetic trees and, thus, both of them are reserved. The topology of the ITS dataset shows that *D. manhanensis* is nested within the monophyletic group comprising *Didymodon epapillatus* J. Kou, X.-M. Shao & C. Feng, *Didymodon mongolicus* D.-P. Zhao & T.-R. Zhang, *Didymodon validus* Limpr., *Didymodon wisselii* (Dixon) D.H. Norris & T.J. Kop. and *Vinealobryum guangdongensis* C. Feng & J. Kou and is sister to *D. obtusus*, but with weakly-supported values. In the combined plastid dataset, *D. manhanensis* is nested within the group including *Didymodon cordatus* Jur. and is sister to *D. daqingii* and *Didymodon anserinocapitatus* (X.J. Li) R.H. Zander, with well-supported values.

Discussion

As indicated by Zander (1993), *Didymodon s. lat.* is heterogeneous and could be profitably split. In our phylogenetic analyses, this genus is polyphyletic and its species can be classified within several well-supported monophyletic clades, which correspond to other phylogenetic studies of the genus (Feng et al. 2022; Jiménez et al. 2022; Zhang et al. in press). Our results reveal a close relationship between *D. manhanensis* and two recently-described species in China: *D. daqingii* and *D. obtusus*. Although the latter two species were considered identical by Sollman et al. (2020), they are not closely related in our phylogenetic analyses, based on both ITS and chloroplast data.

Didymodon manhanensis is distinguished from all congeners by the following combination of diagnostic features: leaves incurved and slightly twisted when dry, spreading when moist, narrowly lanceolate from an ovate base; subulate and fragile leaf apices; distally bistratose leaf margins that are recurved in proximal 2/3–3/4; costal guide cells in 2–3 layers and without ventral stereids, smooth laminal cells and red KOH laminal colour reaction. This combination of characters suggests the placement of *D. manhanensis* in the sect. *Didymodon* (Zander 1978, 1993, 1998). Following the recent revolutionary work on the genus *Didymodon s. lat.* by Zander (2013, 2019), morphologically, it belongs in the amended genus *Didymodon s. str.* Its systematic position in *Didymodon s. str.* was also confirmed by our phylogenetic analyses, based on both ITS and chloroplast data.

Chloroplast data support that *D. manhanensis* is closely related to *D. cordatus* and sister to both *D. daqingii* and *D. anserinocapitatus*. However, *D. manhanensis* differs morphologically from *D. cordatus* by the costa with guide cells in 2–3 layers and without ventral stereids and smooth laminal cells. It differs from *D. daqingii* by the leaves that are narrowly lanceolate from an ovate base, smooth laminal cells and red KOH laminal colour reaction; it differs from *D. anserinocapitatus* by the distally bistratose leaf margins and lack of swollen and deciduous leaf apex (Zander 2007). In the ITS analyses, there is successive branching of clades, including *D. obtusus* J. Kou, X.-M. Shao & C. Feng, *D. manhanensis*, *D. epapillatus* J. Kou, X.-M. Shao & C. Feng, *D. mongolicus* D.-P. Zhao & T.-R. Zhang, *D. validus* Limpr., *Vinealobryum guangdongensis* C. Feng & J. Kou and *D. wisselii* (Dixon) D.H. Norris & T.J. Kop. Amongst these species, *D. manhanensis* is most similar to *D. obtusus*, a species that was recently described from Tibet in China (Kou et al. 2018), but the former can be distinguished from the latter by its narrowly lanceolate leaves from an ovate base and spreading when moist, subulate and somewhat fragile leaf apex and unistratose distal lamina.

There are three species distributed in China that have excurrent costa and smooth laminal cells may be confused with the new species. *Didymodon ditrichoides* (Broth.) X.-J. Li & S. He, a species known from North American, Asia (China) and the Atlantic Islands (Iceland) (Li et al. 2001; Zander 2007), differs from the new species by the unistratose leaf margins, costa with 1–2 layers of guide cells and with 0–1 layer of ventral stereids and yellowish KOH laminal colour reaction (Zander 2007).

Didymodon validus Limpr. can be separated from *D. manhanensis* by the twisted and incurved leaves when dry, unistratose leaf margins, costa with 1 layer of guide cells and with 1–3 layers of ventral stereids and yellowish-green KOH laminal colour reaction (Shuayib et al. 2017).

The lanceolate to long-lanceolate leaves with a widely ovate base, distally bistratose leaf margins, excurrent costa and epapillose laminal cells are likewise found in *Didymodon ochyrarum* J.A.Jiménez & M.J.Cano, a species described from tropical South America (Jiménez and Cano 2019), which may be confused with the new species. However, *D. ochyrarum* can be separated from *D. manhanensis* by its plane leaf margins, marginal basal cells running up the margin forming a distinctly differentiated area of transversely thick-walled cells and yellowish KOH laminal colour reaction.

Taxonomic treatment

Didymodon manhanensis **C. Feng & J. Kou, sp. nov.**
Figs 1–2
Chinese name: 蛮汉山对齿藓

Type. China. Inner Mongolia: Ulanqab City, Manhan Mountain, 40°39'19.2931"N, 112°19'36.3792"E, on soil under the grass, elevation 1417 m, 20 June 2016, *Chao Feng 2016060162* (holotype: NMAC!; isotype: MO!).

Diagnosis. It is distinguished from all congeners by the following combination of diagnostic features: leaves incurved and slightly twisted when dry, spreading when moist, narrowly lanceolate from an ovate base; subulate and fragile leaf apices; distally bistratose leaf margins that are recurved in proximal 2/3–3/4; costal guide cells in 2–3 layers and without ventral stereids, smooth laminal cells and red KOH laminal colour reaction.

Description. Plants medium, growing in turfs, green-blackish distally, brown-blackish proximally. Stems very seldom branched, 0.8–1.6 cm in length, not papillose, transverse section rounded to rounded-pentagonal, central strand developed, sclerodermis present, hyalodermis absent; axillary hairs filiform, of 4–8 hyaline cells, the basal cell brown. Leaves crowded on stem, incurved and slightly twisted when dry, spreading when moist, narrowly lanceolate from an ovate base, constricted just above the base, 1.3–2.3 × 0.43–0.55 mm, distal lamina narrowly channelled ventrally; margins plane distally, recurved in proximal 2/3–3/4 of leaf, entire, distal margins bistratose; apex subulate, somewhat fragile; leaf base ovate, not sheathing, not decurrent; costa stout, tapering distally, 57.5–75 μm wide at base, excurrent as a long, thick subula, not spurred, ventral cells of costa in upper middle part of leaf quadrate or subquadrate, smooth, 4 rows of cells across costa ventrally at mid-leaf, dorsal cells of costa in upper middle part of leaf quadrate or subquadrate, smooth, transverse section semicircular to nearly rounded, epidermis present adaxially and abaxially, not or weakly bulging, ventral stereids absent, guide cells 10–16 in 2–3 layers, 2–4 layers of dorsal stereids, reniform or crescent-shaped, without hydroids; upper laminal cells quadrate

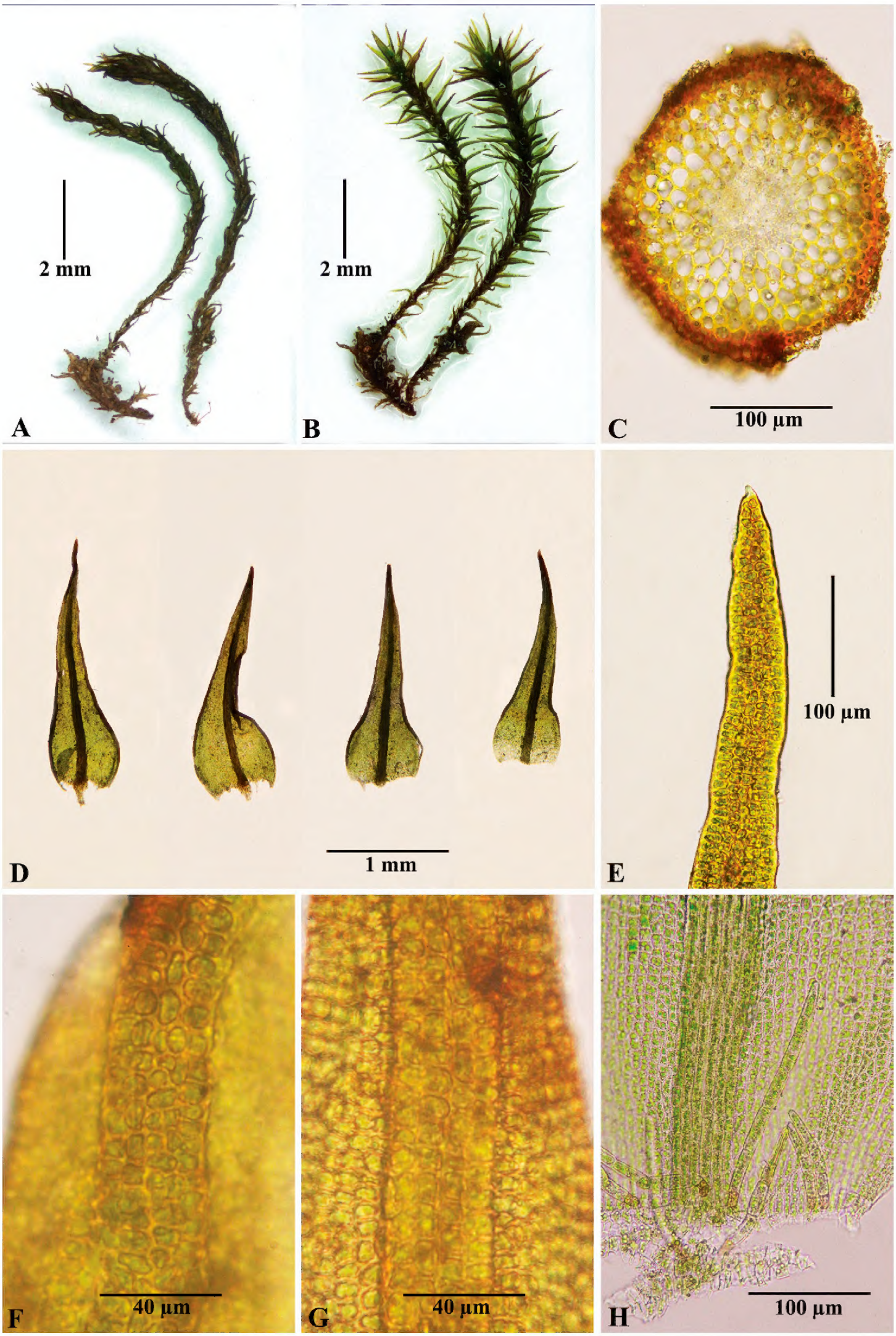

Figure 1. *Didymodon manhanensis* **A** dry plants **B** moist plants **C** cross-section of stem **D** leaves **E** leaf apex **F** upper part of costa (dorsal) **G** upper part of costa (ventral) **H** axillary hairs. Photographed on 21 November 2021 by Chao Feng from the holotype (NMAC!).

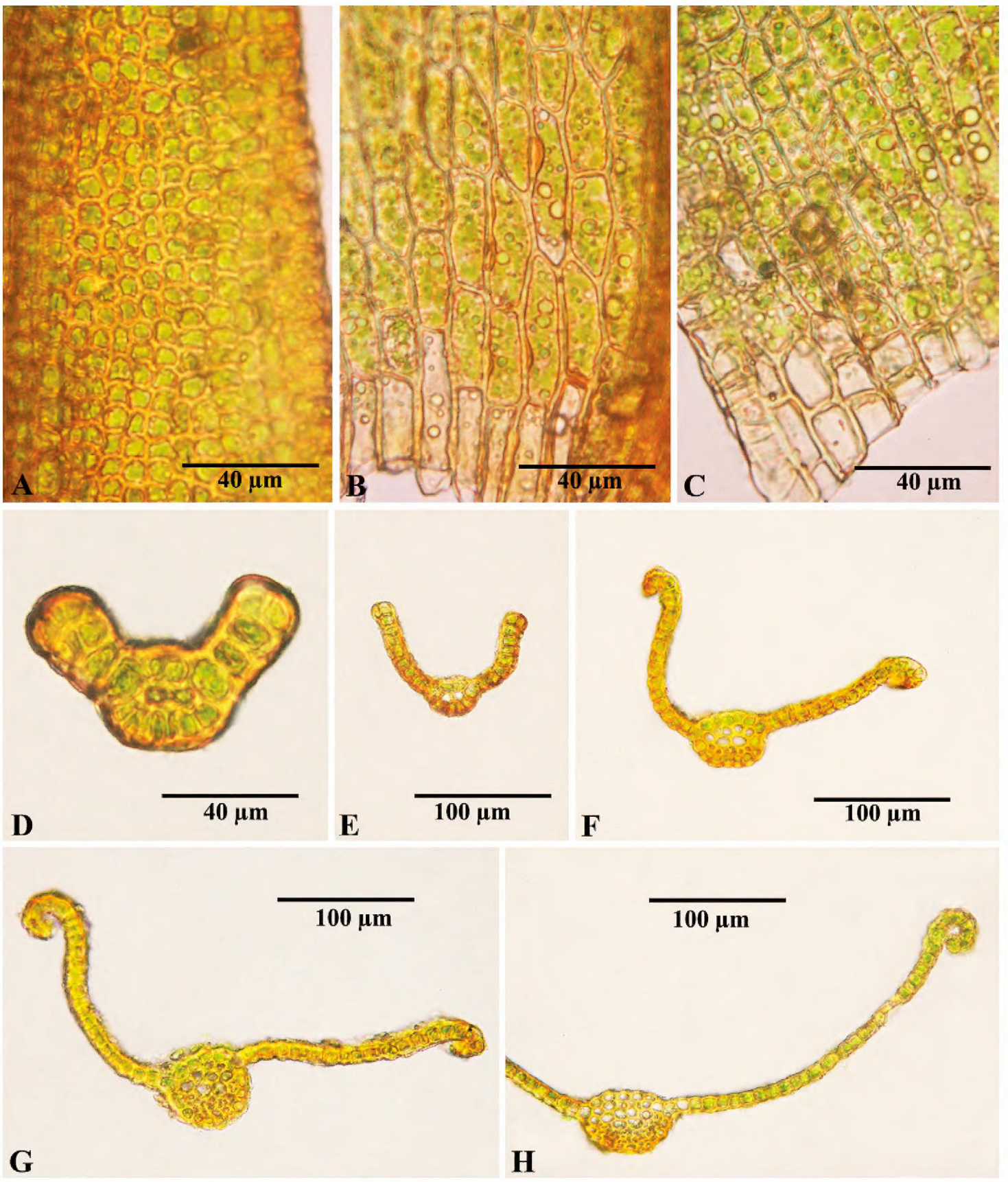

Figure 2. *Didymodon manhanensis* **A** median leaf cells **B** basal juxtacostal cells **C** basal marginal cells; **D–H** cross-sections of leaves, sequentially from apex to base. Photographed on 21 November 2021 by Chao Feng from the holotype (NMAC!).

to rhombic, usually with angular lumens, 7.5–10 × 5–10 µm, smooth, slightly thick-walled, weakly convex on both surfaces, distal lamina unistratose, basal cells weakly differentiated juxtacostally, rectangular, 12.5–37.5 × 5–7.5 µm, thin-walled, smooth; basal marginal cells subquadrate or quadrate, 5–8.75 × 6.25–7.5 µm, with weakly-thickened walls, smooth. Gemmae absent. Dioicous. Sporophytes unknown. KOH laminal colour reaction red.

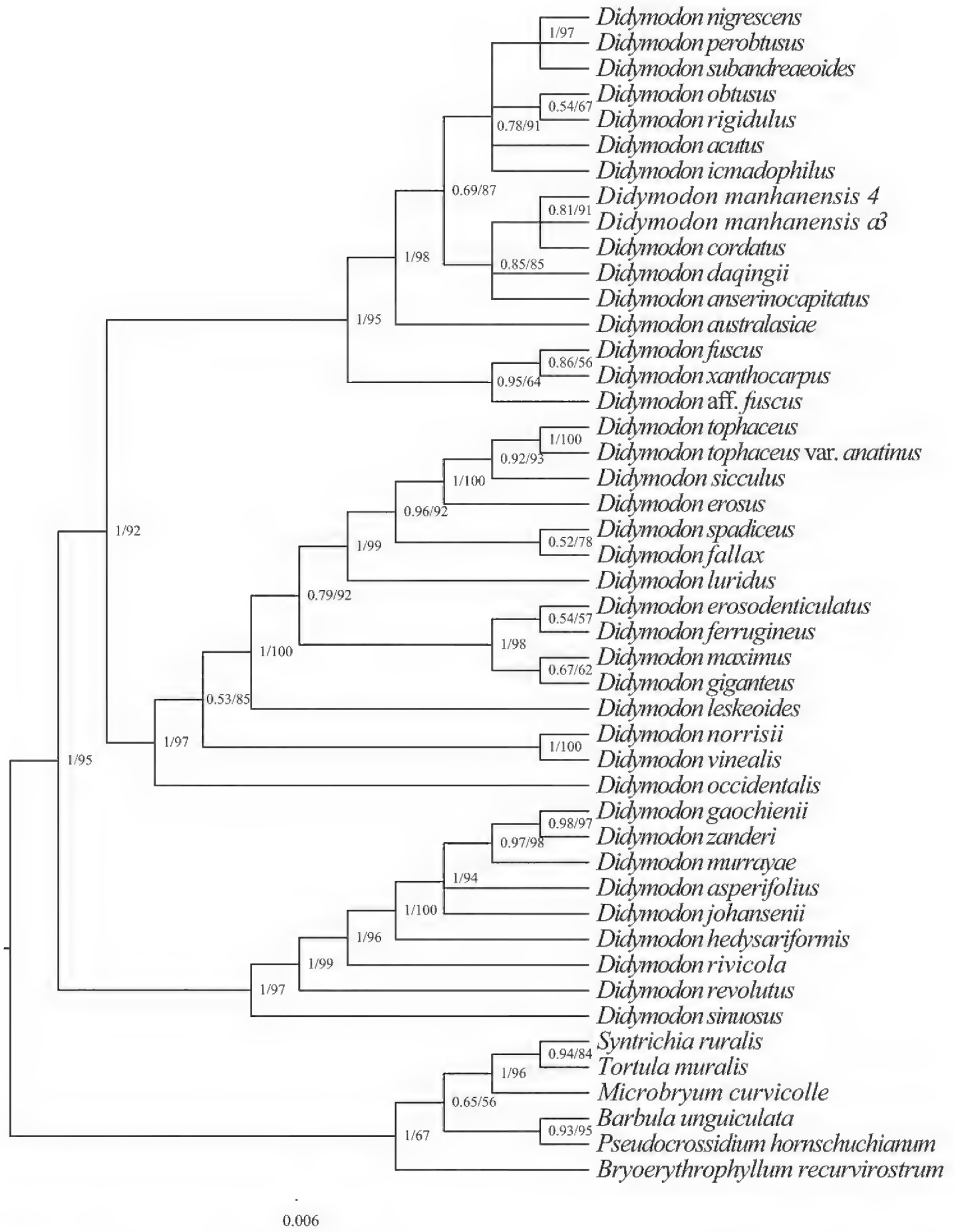

Figure 3. Phylogenetic relationships (50% majority consensus tree) from the Bayesian Inference of the concatenated *rps*4 and *trn*M-*trn*V datasets. Numbers above branches indicate posterior probability from the BI analysis, followed by bootstrap values for the ML analysis.

Additional specimens examined. China Inner Mongolia: Ulanqab City, Manhan Mountain, on soil under the grass, 20 June 2016, Chao Feng 2016060176 (NMAC).

Etymology. The specific epithet refers to Manhan Mountain, the type locality.

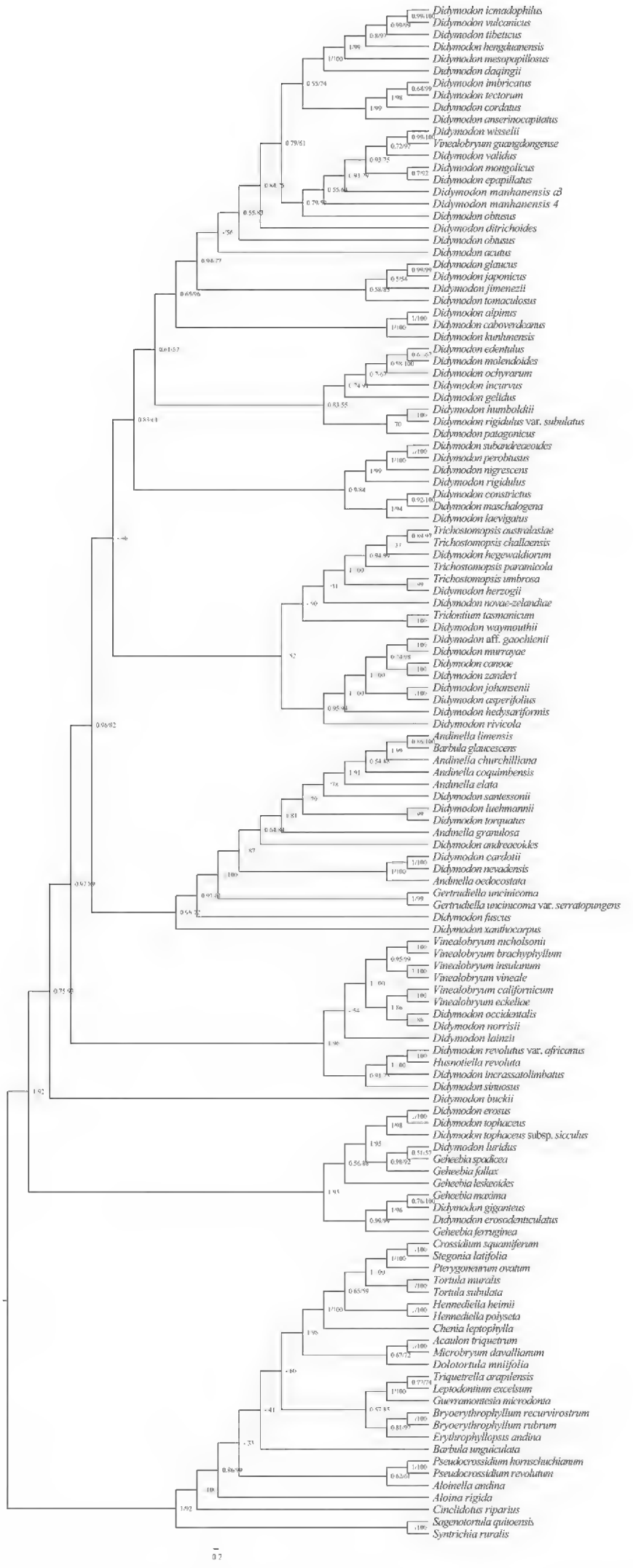

Figure 4. Phylogenetic relationships (50% majority consensus tree) from the Bayesian Inference on the ITS dataset. Numbers above branches indicate posterior probability from the BI analysis, followed by bootstrap values for the ML analysis.

Habitat and distribution. Manhan Mountain is situated in Liangcheng County in the southern section of the Yinshan Mountains in the middle of Inner Mongolia, with an average altitude of approximately 1500 m (Huang et al. 2014). Its soil types are mainly leaching grey, cinnamonic soil (Lyu et al. 2012). The vegetation on Manhan Mountain is typical forest shrub vegetation, including natural forest that consists of *Betula platyphylla* Sukaczev and *Populus davidiana* Dode, plantation that consists of *Larix principis-rupprechtii* Mayr and *Pinus sylvestris* var. *mongolica* Litv., natural shrubs that consists of *Ostryopsis davidiana* Decne., *Spiraea salicifolia* L. and *Rosa davurica* Pall. and the herbaceous plants including *Stipa bungeana* Trin., *Cleistogenes squarrosa* (Trin.) Keng, *Lespedeza bicolor* Turcz., *Carex* spp. and *Leymus chinensis* (Trin.) Tzvelev (Zhang et al. 2017; Li et al. 2021). *Didymodon manhanensis* is currently known only from the type locality at the foot of the Manhan Mountain, north-western Liangcheng County, Inner Mongolia, China, growing on soil under the grass.

Key to species morphologically similar to *D. manhanensis*

1	Leaf apices apically swollen as a propagulum	***D. anserinocapitatus***
–	Leaf apices not swollen, usually evenly narrowing	**2**
2	Cells on the upper ventral surface of the costa elongate	***D. wisselii***
–	Cells on the upper ventral surface of the costa quadrate	**3**
3	Laminal cells smooth	**4**
–	Laminal cells papillose	**10**
4	Costa with 2–3 layers of guide cells and without ventral stereids	**5**
–	Costa with 1 layer of guide cells and with ventral stereids	**6**
5	Leaves patent to spreading when moist, leaf lamina bistratose	***D. obtusus***
–	Leaves spreading when moist, leaf lamina unistratose	***D. manhanensis***
6	Costa percurrent or ending before the apex	7
–	Costa long-excurrent	**8**
7	Leaf margins bistratose near apex	***D. epapillatus***
–	Leaf margins unistratose	***D. mongolicus***
8	Plants flagellate, leaves linear-lanceolate	***D. ditrichoides***
–	Plants thickly leaved, leaves short-lanceolate to long-lanceolate	**9**
9	Leaves appressed when dry	***D. acutus***
–	Leaves twisted or incurved when dry	***D. validus***
10	Leaf margins plane	***D. tibeticus***
–	Leaf margins recurved	**11**
11	Costa without ventral stereids	**12**
–	Costa with ventral stereids	**13**
12	Costa excurrent	***D. daqingii***
–	Costa ending below apex	***D. imbricatus***
13	Marginal basal cells forming a distinctly differentiated area of smooth and transversely thick-walled cells	***D. hengduanensis***
–	Marginal basal cells not forming a distinctly differentiated area	**14**

14	Distal laminal cell superficial walls thicker than the internal walls	***D. mesopapillosus***
–	Distal laminal cell superficial walls of same thickness as the internal walls	**15**
15	Laminal cells with low papillae over the transverse walls, which reach the two adjacent cells	**16**
–	Laminal cells with papillae situated over the lumina	**17**
16	Leaves spreading when moist	***D. guangdongensis***
–	Leaves erect to patent when moist	***D. vulcanicus***
17	Leaf margins recurved in proximal 1/4–3/4	***D. icmadophilus***
–	Leaf margins strongly recurved or revolute to near apex	**18**
18	Leaf base squared in shape, costa slender	***D. tectorum***
–	Leaf base usually ovate in shape, costa stout	***D. cordatus***

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